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The TCR Triggering Puzzle

Minireview

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T cell antigen recognition requires the binding of the T cell antigen receptor (TCR) to a complex between an antigen (usually peptide) and an MHC molecule (pep-MHC). This leads to a series of signaling events collectively referred to as TCR triggering, which is mediated by a group of TCR-associated transmembrane signaling molecules, the CD3 complex. Although it has been intensively studied, we still do not have satisfactory answers to two fundamental and related questions concerning TCR triggering. First, by what mechanism does TCR binding to pep-MHC result in triggering? Second, what binding property governs whether a particular TCR/pep-MHC interaction will lead to triggering? I discuss both of these questions here with special reference to two important recent papers (Baker and Wiley, 2001; Kalergis et al., 2001), the former being published in this issue of *Immunity*.

Many ideas concerning TCR triggering have been influenced by insights gleaned from other receptor systems, particularly receptors that, like the TCR, signal by stimulating tyrosine phosphorylation. However, the TCR/peptide-MHC interaction has unique features that should be borne in mind when considering possible answers to the questions posed above. First, the pep-MHC binding site on a TCR is generated in a quasi-random manner, and there is enormous diversity in the fine structure of interacting TCR and pep-MHC binding surfaces. Second, following crystallographic analysis of only a handful of pep-MHC complexes it is already clear that the orientation in which different TCRs engage pep-MHCs, although constrained to a certain extent, is quite variable (Garcia et al., 1999; Hennecke and Wiley, 2001). For example, the angle of engagement measured around the long axis of the TCR/pep-MHC complexes studied thus far differs by up to 35° (Hennecke and Wiley, 2001). Consequently, the structure of TCR/pep-MHC complexes is also likely to vary. Finally, T cells are required to, and are able to, recognize exceptionally low levels of specific pep-MHC on cells (see Kimachi et al., 1997, and references therein); indeed, a single pep-MHC complex on a target cell may be sufficient.

Triggering Models

The models that have been proposed for TCR triggering can be divided into three groups depending on whether they invoke, as mechanisms of signal transduction, binding-induced multimerization, binding-induced conformational change, or neither (Figure 1). *Multimerization models* propose that ligand engagement brings together two or more TCR/CD3 complexes, which results in signaling through either induced proximity of associated signaling molecules or, as suggested more recently (Harder, 2001), the partitioning of aggregated TCR/CD3 complexes into lipid rafts that are themselves enriched in signaling molecules. Such models require the simultaneous engagement of at least two adjacent TCRs by pep-MHC and so fail to account for the fact that TCR triggering can occur at, and indeed is most efficient at (Lanzavecchia et al., 1999), very low densities of pep-MHC. A possible solution to this difficulty was provided by two studies using soluble TCR and pep-MHC, which suggested that, following pep-MHC binding, TCR/pep-MHC complexes oligomerize (Reich et al., 1997; Alam et al., 1999). The mechanism proposed was that, upon binding, the TCR or TCR/pep-MHC complex acquires the ability to bind directly to another TCR or TCR/pep-MHC complex. However, the structural diversity of TCR/pep-MHC complexes noted above raises doubts as to whether binding-induced self-association can be a general feature of TCR/pep-MHC interactions. Furthermore, individual TCR/pep-MHC complexes are quite heavily glycosylated and surrounded by CD3 and coreceptor glycoproteins (Rudd et al., 1999), which would seem to preclude *direct* physical association of two or more TCR/pep-MHC complexes. These doubts are supported by a study reported in this issue that looked for evidence of binding-induced self-association in two soluble TCR/pep-MHC systems (Baker and Wiley, 2001). Using several sensitive techniques, including those used by Reich et al. (1997) and Alam et al. (1999), they convincingly rule out self-association in these systems. Taken together with other studies (e.g., Willcox et al., 1999) that find no evidence of the complex binding kinetics that should be observed with self-association, this suggests that binding-induced self-association is not a general feature of TCR/pep-MHC interactions and is therefore unlikely to be the mechanism of TCR triggering.

Conformational change models typically propose that, upon binding to pep-MHC, a TCR undergoes a conformational change that is somehow transmitted to the associated CD3 signaling machinery. The difficulty with these models is that they require, implausibly, that there is a conformational change in the TCR coupled to binding that is conserved in all TCRs in the face of enormous and semirandom variability in the TCR/pep-MHC binding interface. The fact that a number of recent structural studies have failed to reveal any evidence for conformational changes in the TCR other than adjustments at the binding interface would seem to rule out such models (Garcia et al., 1999; Hennecke and Wiley, 2001). A more plausible conformational change model has been proposed recently that is compatible with the available structural data (Ding et al., 1999). According to this *dimer conformational change model*, two TCRs are present as a preformed dimer (i.e., two $\alpha\beta$TCRs), and simultaneous binding of each TCR to specific pep-MHC alters the relative orientation of the two TCRs. Interest in the latter model has been stimulated by evidence for the existence of preformed TCR dimers on the T cell surface (Fernandez-Miguel et al., 1999). While

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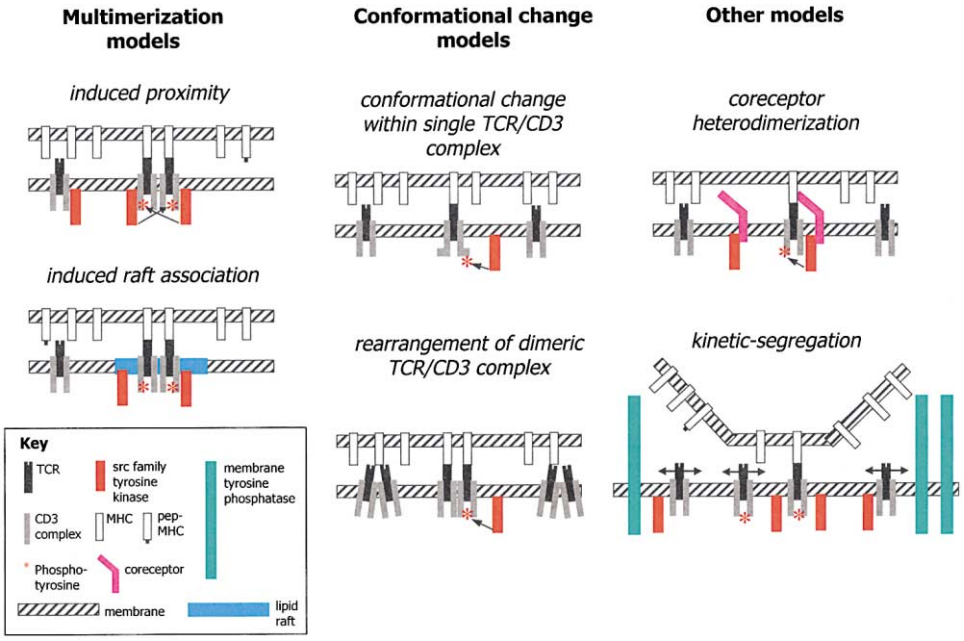

Figure 1. TCR Triggering Models

The key early event in all the illustrated models is tyrosine phosphorylation of CD3 chains. In the kinetic-segregation model, it is proposed that tyrosine phosphorylation of *unengaged* TCR/CD3 in the close-contact regions is transient because unligated TCR/CD3 will soon diffuse out of the close-contact region and be exposed to tyrosine phosphatases. In contrast, *engaged* TCR/CD3 is "held" within this region, enabling subsequent triggering steps to proceed.

the dimer adjustment model, like multimerization models, requires the simultaneous engagement of two adjacent TCRs, the presence of preformed TCR dimers should significantly increase the likelihood of this event.

The evidence and arguments outlined above have increased interest in models of TCR triggering that do not invoke conformational change or multimerization (Figure 1). One such model postulates that triggering results from the *heterodimerization* of the TCR with the CD8 or CD4 coreceptors following binding to the same pep-MHC complex, thereby inducing proximity between the TCR/CD3 complex and coreceptor-associated signaling molecules. Support for such a model was provided by the observation that monovalent soluble pep-MHC can trigger T cells provided that they express CD8 (Delon et al., 1998). However, coreceptor heterodimerization cannot be a general mechanism since TCR triggering can occur in cells entirely lacking coreceptors. The *raft association model* (not shown in Figure 1) postulates that TCR engagement leads to association of that TCR/CD3 complex with lipid rafts (Lanzavecchia et al., 1999). However, no convincing mechanism has been proposed to explain how engagement of a single TCR/CD3 leads to raft association. The *kinetic-segregation model* (Davis and van der Merwe, 1996; van der Merwe et al., 2000) proposes that pep-MHC binding induces triggering by tethering the TCR/CD3 complex within a zone of close membrane contact in which tyrosine phosphorylation is favored. While the TCR/CD3 complex is held in this zone, the sequence of phosphorylation and phosphorylation-dependent steps required for TCR triggering can proceed. The model proposes that tyrosine phosphorylation is favored because the formation of a close-contact zone is accompanied by exclusion or segregation from the zone of membrane tyrosine phosphatases, many of which, like CD45 and CD148, have large ectodomains that could drive exclusion. It is important to stress that the segregation proposed here is on a much smaller scale than the large-scale segregation of cell-surface molecules characteristic of the immunological synapse that forms between an activated T cell and APCs or target cells (Monks et al., 1998; Grakoui et al., 1999; Stinchcombe et al., 2001). The latter follows and depends upon TCR triggering and so cannot be the mechanism of TCR triggering (van der Merwe et al., 2000). It has been proposed instead that large-scale segregation is required for, or the result of a process required for, polarized secretion by T cells toward antigen-presenting or target cells (Davis and van der Merwe, 2001).

Binding Properties

What feature of a TCR/pep-MHC interaction determines whether pep-MHC binding will result in TCR triggering? The notion that the nature of the response is determined by the particular structural change induced in a TCR can probably be ruled out, for two reasons. First, the highly variable structure of TCR/pep-MHC interfaces makes it implausible that the same conformational changes are induced in all TCRs in response to ligand binding. Second, there is no evidence of structural changes in TCR/pep-MHC complexes that correlate with the functional outcome of binding (Garcia et al., 1999; Hennecke and Wiley, 2001). Particularly convincing are the demonstrations that the structure of a given TCR is essentially the same whether bound to agonist, superagonist, or antagonist pep-MHC (Ding et al., 1999; Degano et al., 2000).

It would seem more plausible that the TCR response is determined by a binding property related to binding strength, such as affinity or half-life. It is unlikely for two reasons that the affinity per se is the key property. First, differences in affinity can only be detected by the T cell as a difference in the number of engaged TCRs, which fails to account for situations in which only a few pep-MHC complexes are sufficient to trigger a T cell. Second, it is difficult to envisage how the TCR could discriminate between ligands with small differences in affinity, as is the case. More attractive is the notion that the T cell response is dependent on the half-life of the TCR/pep-MHC interaction. By coupling a dependence on half-life with a requirement for several consecutive signaling steps for TCR triggering, the TCR can discriminate very effectively between pep-MHCs that differ only slightly in their TCR binding half-lives (McKeithan, 1995).

A number of studies have measured the affinity and kinetics of interactions between soluble forms of TCR and pep-MHC and attempted to correlate these properties with the functional outcome of the interaction. While there is a crude correlation between binding strength as measured by affinity or half-life and T cell response, this correlation tends to break down when TCR/pep-MHC interactions with smaller (2- to 5-fold) differences in affinity or half-life are compared. One possible explanation for this is suggested by the recent study by Kalergis et al. (2001). They measured the dissociation half-life of pep-MHC class I tetramers from T cells and correlated these with functional responses. In the two systems studied, they found that TCR/pep-MHC interactions with the longest half-lives did not give the best functional response. Importantly, a TCR mutation that reduced the half-lives of "long half-life" pep-MHCs actually enhanced TCR triggering. Thus, there appears to be an optimal TCR/pep-MHC half-life or "dwell time" with longer and shorter half-lives correlating with poorer responses. This was predicted by, and provides support for, the serial triggering model (Lanzavecchia et al., 1999), which postulates that a certain threshold number of TCRs needs to be engaged for T cell activation, and that each pep-MHC can serially engage multiple TCRs. It follows that pep-MHCs that engage TCRs with a half-life longer than that required for triggering will be less effective agonists because they will trigger fewer TCRs in a given time period.

There remain, however, data that are not explained by the optimal dwell time/serial triggering model. For example, it has been observed that TCR/pep-MHC interactions with very similar half-lives can have very different functional outcomes (Baker et al., 2000, 2001). One possible explanation for these discrepancies is that the binding half-life measured in solution (where at least one of the molecules is soluble) does not accurately reflect the half-life of the TCR/pep-MHC interaction at the cell-cell interface. One crucial difference in the interactions between soluble molecules and between their membrane-tethered counterparts is that in the latter case the interactions are subjected to mechanical stress, i.e., there will be some traction force applied to the TCR/pep-MHC bond. This force arises from one or more of several sources including thermal fluctuation in the membranes, repulsive forces between the membranes, and movement of the T cell relative to the APC or target cell. Crucially, the half-life of an interaction decreases when subjected to mechanical stress, but the extent to which it decreases will vary between interactions. Where studied, the binding property that correlates best with the mechanical strength of a bond was the activation enthalpy (Leckband, 2000). It may be significant therefore that TCR/pep-MHC interactions have a remarkably high activation enthalpy (Boniface et al., 1999; Willcox et al., 1999), suggesting considerable mechanical strength. This is consistent with the observation that T cells can extract and internalize engaged pep-MHC from target cells (Huang et al., 1999) and tend to leave behind TCRs when forcibly detached from planar bilayers presenting pep-MHC to which they bind (Dustin et al., 1996). These observations suggest that TCR/pep-MHC half-lives at the cell:cell interface may not always correlate with half-lives measures in solution, and raise the question as to whether the mechanical strength of a TCR/pep-MHC interaction, by determining the half-life under mechanical stress, is an important determinant of TCR triggering.

Conclusions

Despite extensive efforts, the mechanism of TCR triggering remains poorly understood, and there are a number of competing models. There are major difficulties with more traditional models such as those postulating binding-induced conformational change of the TCR or binding induced-multimerization. Newer models, such as the *dimer conformational change*, *raft-association*, and *kinetic-segregation models,* have been proposed that are compatible with the available data, but they have yet to be rigorously tested.

There is more consensus on which property of TCR/pep-MHC interactions determines the outcome of binding. Structural consideration and data have ruled out ligand-specific conformational changes as a determinant. The available data are broadly consistent with the hypothesis that there is an optimal half-life or dwell-time for TCR/pep-MHC interactions. Some data are not consistent with this, possibly because the TCR/pep-MHC half-life is different under physiological conditions in which the interaction is subjected to mechanical stress. Measurements of the mechanical properties of TCR/pep-MHC interactions and direct measurement of the kinetics of membrane-tethered TCR/pep-MHC interactions are needed to clarify this question.

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